WEBVTT

00:00:00.900 --> 00:00:24.230 It's my pleasure to now introduce the second session of this symposium will be starting with the topic of taming the tumor microenvironment ecosystem and our first speaker will be doctor. Greg Dolgoff Doctor del golf comes to us from the University of Pittsburgh, where he is an assistant professor in the Department of analogy.

00:00:32.710 --> 00:00:34.820 All right.

00:00:37.920 --> 00:00:38.790 Cool.

00:00:40.180 --> 00:01:11.290 Can you hear me alright we're good alright? Thank you very much? I'm honored to be to be invited to the symposium, it's I felt very welcomed since since I arrived yesterday. So I'm going to talk to you today. We're going to switch gears, not a whole lot of sexy image Ng or anything else like that. But we're going to talk about something that's generally think we're now becoming a little bit more generally appreciated but the fact is actually goes back a century or 2.

00:01:11.290 --> 00:01:23.980 Understanding the role of metabolism, both in the generation of an immunosuppressive tumor micro environments and how those factors play with immune cells?

00:01:24.960 --> 00:01:41.040 These are these are my disclosures. The biggest one is I'm a founder in scientific advisor of this company TMS. We will not be. I won't be discussing anything really related to that work in this talk, but I just in the interest of full transparency.

00:01:41.750 --> 00:02:11.760 So the reason why we're excited about this about this question is that we know that the process of engaging in immune response and this is work that goes back a couple of decades now is metabolically demanding process so naive T cell right. These cells that sit and wait rights. They need to be able to persist potentially for a lifetime, having never seen their target. Antigen so they have to be extremely quiescent, but then upon activation need to enter.

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NOTE Confidence: 0.901903688907623

00:02:11.760 --> 00:02:42.060 Into an extremely proliferative phase in order to generate a clonal army now, so there's been a lot of excitement around the role of how does a T cell? How did the TCL fuel that response? What we now know? Is it is T cells go from naive to early affect ourselves to being able to infiltrate into a target tissue and then scan and destroy that they have very, very different metabolic needs. But more importantly after an immune response is. Over a cell needs to be able to go back into that.

NOTE Confidence: 0.912728846073151

00:02:42.060 --> 00:03:13.050 Long lived, Quiessence states, but ready to see Anagen again. And so we now know that memory T cells are characterized by having a high degree of mitochondrial mass and this value that we call spare respiratory capacity, which is kind of like parking your mitochondria and reserve and keeping them in this healthy healthy metabolically primed space. And so we've been really trying to understand how to T cells behave when they're stimulated in those dearth environments.

NOTE Confidence: 0.856121420860291

00:03:13.050 --> 00:03:43.480 And that's really how things come so this is one. One cool image that I get to show you today and this is this is a tumor section where we are able to mark areas of high glucose uptake using a fluorescent glucose tracer. This is a one amino sci-fi glucose, which is kind of imagine it like FDG, but its fluorescence and this is a marker and antibody staying for a marker called P Magnetism is a radio sensitizer old radio size doesn't work very well.

NOTE Confidence: 0.887016832828522

00:03:43.480 --> 00:04:16.450 But it does Mark Hypoxic regions very well. You can come back with an antibody and staying for it. The reason why I'm going into details. 'cause this marker this PM and it is all marker is going to come up again and again throughout the talk so you can see in there, too or micro environments. It's not this kind of like hypoxic core surrounded by healthy tissue like the way that we see in pictures, but rather their zones of hypoxemia their zones of high glucose uptake and T cells are kind of smattered around there. There's an experience of all these different metabolic parts. So we got excited about this because cancer is driven by metabolic deregulation if you believe Autovon Warburg.

NOTE Confidence: 0.902241289615631

00:04:16.450 --> 00:04:47.140 He thought that was the reason that you have cancer cells. However, we do know that that includes increased nutrient uptake and this kind of deranged glycolytic and oxidative metabolism. And that creates landscape. That's distinct from normal tissues. And so the tumor. Microenvironment is driven by that metabolic derangement generates both a Los

of a central nutrients things like glucose and oxygen, but also a buildup of toxic byproducts. And so we wanted to really ask the question is that in the patient population is this a thing.

NOTE Confidence: 0.88872766494751

00:04:47.140 --> 00:05:18.410 Is it are all tumors turned up to 11 or is it different from the patient patient to patients and so this is unpublished data. The only piece of published data to talk about the the other 2 stories are actually are nascent works. But we hypothesize that we could get insight into the tumor microenvironment by metabolically profiling. The tumor cells and so one of the ways that we do this is using an instrument called the seahorse gives you a real time view of the metabolic proclivities.

NOTE Confidence: 0.85374927520752

00:05:18.410 --> 00:05:48.700 Who sells so how oxidative they are or how glycolytic they are and this is these are the readout so we're going to use in this in a kind of 2 dimensional plot and so we basically did at first, and in Pittsburgh. We were very heavily with the Melanoma program. And so we started by just kind of thawing out all of the human cell lines that we could find in the freezer and we put them into the sea horse in the same media asking them? What kind of metabolism. They perform and we can get these 2 different readouts and here's where the data.

NOTE Confidence: 0.889207243919373

00:05:48.700 --> 00:06:21.570 But you can see is that it's kind of all over the place you have some cells that are truly hypermetabolic like this, a 375 cell line that everybody loves and you have other cell lines that are not so are not so hypermetabolic. They may only do regulate one type of metabolism. Like this M308 Line is solely oxidative and does actually no appreciable glycolysis. That kind of fermentation of glucose into lactic acid and you have other ones that do kind of the opposite like this M255 Line and so there was a lot of heterogeneity in these cells that have been in culture for decades, so.

NOTE Confidence: 0.876234114170074

00:06:21.570 --> 00:06:49.140 We wanted to understand a little bit more about what kind of environments. These tumors might produce so we did what most terminologists are loath to do, which is we put it into a unit efficient animals, So what we do is we let those guys form a tumor in an NSG mouse and then we infuse them with that payment. It is all molecule that molecule that could mark hypoxia and we asked could we guess can, we see a difference in the generation of hypoxemia based on the tumor cell metabolism.

NOTE Confidence: 0.862813889980316

00:06:49.880 --> 00:07:21.050 And here's some of the data without hypoxia probe antibody that the thing that this molecule is telling about before is that if the cell was characterized by having a high oxphos signature. Those cells

those tumors that form data threshold size. So all of these are the same size tumor produce different levels of hypoxia, but this guy down here. This M255 Line, which does far less oxidative. Metabolism didn't really produce a lot of hype oxium so it seems kind of like the oxygen has to go somewhere.

NOTE Confidence: 0.881420910358429

00:07:21.050 --> 00:07:52.720 But we were trying to link the oxphos signature of the tumor cell with a generation of hypoxia in vivo and we think we've done this here and I'll show you some data in competent models that we think we think that we're making some strides here, so that's of course in cell lines could we do the same thing from patient samples. The beauty of this seahorse instruments is that you don't need the millions of cells that you used to put into a Clark electrode. You could do this from very small numbers of cells and so we hypothesize we could do this from biopsies and so all this is going to be from.

NOTE Confidence: 0.871837913990021

00:07:52.720 --> 00:08:23.690 Metastatic Melanoma tumor biopsy is not we have a Sentinel Lymph node project as well. But I'm not going to talk about that today and so we have this IRB approved Immunol Metabolic Profiling Protocol, where we take a section of the tumor. We do some embedding and do Codex. We sort away. The leukocytes so all the CD 45 positive till we have sorting panel and then we do all kinds of cool stuff and then we take the tumor cells and subject them to a metabolic profile DirectX vivo.

NOTE Confidence: 0.881720364093781

00:08:24.500 --> 00:08:54.510 We can do this with Melanoma because they like plastic we try to do this with other with other cancer models and we can talk a little bit about that offline so we go back to the same kind of plot oxphos versus glycolysis. What we found is the same heterogeneity in the patient population that we observed in the cell lines that some folks were characterized by hypermetabolic tumors while others had solely oxidative or solely glycolytic tumors and so I put in this up because as an immunologist. I liked a subset things and so we started subsetting these patients.

NOTE Confidence: 0.89422607421875

00:08:54.510 --> 00:09:24.620 Into these groups based on how energetic or Singley metabolically active their tumors were, and then because of course. This was patient samples. We could then profile. The matched PBL until that we got from each of these individual patients and so when we do. That kind of work we create were able to get a sense of linking the tumor cell metabolism to the T cell metabolism. But not in the same environment member. We've sorted away. Those T cells so in order to account for any differences and T cell receptor.

NOTE Confidence: 0.871145367622375

00:09:24.620 --> 00:09:54.830 Expression and things like that. We just did a very brief chemical restimulation to ask what kind of cytokines could these T cells make if if they were given a chemical activation and if you look at T cells that we isolated from those very energetic tumors and we again re stimulated them in isolation, which you can see is kind of classic tumor induced immune suppression is that PDL from these these patients were competent to make TNF interferon gamma, but the T cells from the tumor.

NOTE Confidence: 0.870657563209534

00:09:54.830 --> 00:09:56.430 We're far less competent to do so.

NOTE Confidence: 0.856245160102844

00:09:57.490 --> 00:10:13.580 And again if you look at T cells that came from those more indolent tumors. Those tumors that didn't really do regulate either their oxphos signature or the glycolysis signature those T cells. Look just fine. They really were no different from the circulating T cells from the same patients.

NOTE Confidence: 0.88579922914505

00:10:14.120 --> 00:10:44.170 But when we started looking at those individuals that's when things got really, really, really interesting is it of T cells came from these hyper glycolytic tumors. They weren't that bad, especially when they were isolated away from the tumor cell and re stimulated is that their fate hadn't really changed and in fact, they were there was statistically. No difference between the glycolytic tumors and the quiet and tumors. But it was really coming from this hyper oxphos signature that rendered the T cells less polyfunctional so.

NOTE Confidence: 0.901650667190552

00:10:44.170 --> 00:10:54.790 IDK spoke being in that highly oxidative environments change the fate of those cells such that they were they were less that they were less functional. Even when they are no longer in that environment.

NOTE Confidence: 0.904953062534332

00:10:55.350 --> 00:11:26.470 And so of course, every one of those patients is individual with their own immune system their own tumor, so could we assess the individual contributions of these metabolic pathways in a more controlled system could be reversed translated and so we used we used Marcus mice. Instead, the original plan was to make single cell clones and that there was going to metabolic heterogeneity of individual clones and was going to be this really cool experiment and we ended up wasting a lot of time because by the time you took the single cells out and he made clones.

NOTE Confidence: 0.893019378185272

00:11:26.470 --> 00:11:57.300 Everything was turned up to 11, the process of the in vitro culture was wouldn't give us the heterogeneity that we really wanted so. Instead, what we did is we took a single clone that we made from a Melanoma

that forms. On on this animal and which we call clone 24 and this clone was extremely energetic completely insensitive to immunotherapy and very, very, very aggressive, but instead then targeted a single metabolic pathway in those cells.

NOTE Confidence: 0.909500420093536

00:11:57.310 --> 00:12:28.670 And we chose 2 obvious ones one was the glucose transporter. SLC 2A, one to prevent that glycolytic metabolism. And another one was this in this molecule called N duffs 4, which is a nuclear encoded component of mitochondrial complex one that first step in the electron transport chain that drives oxidative metabolism. All the things that you try to forget about from undergraduate biochemistry. Sorry it's all going to come back to haunt you now here's what happens if you take the control sells their highly highly energetic.

NOTE Confidence: 0.881551802158356

00:12:28.670 --> 00:12:58.700 If you knock down the glucose transporter you then recreates the solely oxidative tumor cell line. And if you knock down that mitochondrial complex. One you create solely glycolytic tumor cells. This all RNA interference. We used crisper as well. But there are some caveats, which we can discuss offline, but now these are all derived from the same single cell so now we can put them into tumors aren't sent to Animals and that's what we do, if we take these tumors Interestingly enough. This isn't very aggressive cell line.

NOTE Confidence: 0.898503363132477

00:12:58.720 --> 00:13:09.280 And so it doesn't matter the cells always form tumors, which was a little bit of a surprise to us. But in the absence of any immune stimulation the cells all form tumors at the same rates.

NOTE Confidence: 0.897366285324097

00:13:09.840 --> 00:13:27.010 But it was when you look inside. The tumor that things changed and the big shift that we saw is that if you not doubt the glycolytic metabolism of the tumor. Nothing changed nothing. Immuno logic changed the cells were the states of the T cells that were inside was similarly poor.

NOTE Confidence: 0.874415516853333

00:13:27.520 --> 00:13:50.790 However, if you inhibited the oxidative metabolism of the tumor cells lower that oxphos signature. the T cells had a more favorable profile. They were we had less terminally exhausted T cells. The cells that are PD1 high and expressing multiple Co inhibitory molecules and we had more of the PD one intermediate cells. Those stem like cells that are thought to be the true targets of PD one blockade.

NOTE Confidence: 0.85648101568222

00:13:51.550 --> 00:14:02.340 If you don't like this kind of nomenclature. That's fine. We also just re stimulated them and showed that if T cells came from this less oxidative tumor. They were more competent to make cytokines when sorted away and re stimulated.

NOTE Confidence: 0.86227685213089

00:14:02.950 --> 00:14:34.140 So this is interesting, I guess looking at kind of subtle shifts in the tumor microenvironment. But what is the tumor microenvironment actually look like and here here these same hypoxia imaging that was talking about before these are whole tumors stitched together. You can see that when you knockout that oxidative metabolism. You truly knockout hypoxia. If you knockout. The glucose metabolism. You actually drive hypoxia, which is a very important pipe points. So when you knockout one fuel source. The tumor cell has to use the other So what you can find is that these are not standing artifacts. These are actually depots.

NOTE Confidence: 0.872831463813782

00:14:34.140 --> 00:15:04.150 Of T cells that are stuck on the outside of these hypoxic regions. But when you lack that hypoxia staining the T cells are able to penetrate deeper into the tumor bed and far more importantly, if you take these mice and then put them on immunotherapy. This is where the key comes out is that the glycolytic metabolism. If you knock it out doesn't make any bit of difference. Those T cells. Those tumors do not respond to anti PD one. However, if you knockout. The oxidative pathway. You then start to see responses to PD one blockade so this is pretty exciting this linked the oxidative metabolism, the tumor.

NOTE Confidence: 0.86462014913559

00:15:04.150 --> 00:15:23.810 With the inability to respond to checkpoint blockade and we're not the first people to talk about this. In fact, RNA sequencing of tumors and that they have innate resistance to immunotherapy. The number 2. Gene ontology list is hypoxia. So we know this, but it was nice to confirm this any tumor cell intrinsic way.

NOTE Confidence: 0.891272246837616

00:15:24.440 --> 00:15:56.230 So now we went back to the patient samples and we asked the question could we then do our oxphos versus glycolysis signature and ask some questions about whether those patients are going to respond to immune based therapy. So this is all assessment of that tumor cell metabolism. But this is in a cohort of people of patients. All metastatic Melanoma about to go on either niveau or Pembroke Monotherapy and this is all pretreatment biopsies. And if you then look at this look at this bivariate plots and you color it based on who goes on to progress.

NOTE Confidence: 0.853418052196503

00:15:56.230 --> 00:16:15.590 You can see that if the tumor cell hasda regulated oxphos signature. It is that patient is more likely to progress on anti. PD one here are the data broken down by non responder versus responder. And here here's the overall survival data. Whether or not to sell as an ox. Phos high signature or an ox phoslo signature.

NOTE Confidence: 0.885952353477478

00:16:16.360 --> 00:16:46.990 Even in progress, even in patients that respond. The duration of response. We could find also would stratify with the aux file signature and we've seen some similar data to this being shown in a Cup with a couple different groups, namely Mike Davies Group at MD Anderson as well as some work from my current also in Houston. So we think this is an important idea of understanding what the tumor cell metabolism is and how does that relates to immunotherapy response now?

NOTE Confidence: 0.845203399658203

00:16:46.990 --> 00:17:18.160 That's a lot of work taking these digestions getting out the tumor cells, putting them into the Sea Horse. It's exciting from an academic perspective, but the fact that matter is could we just look for hypoxia and that's one of the things that we did. We used we are now treating people with PM and it is all it has and I? Indeed, it's a safe tracer that you can use, however you need to pulse patients. You can't look retrospectively. But we have looked at hypoxia by a pretty standard chemistry antibody.

NOTE Confidence: 0.832150340080261

00:17:18.160 --> 00:17:34.890 Carbonic anhydrase 9 it's a target of hypoxic signaling it's not true hypoxia, but it's close and this is if we get strong membranous staining for CA 9 were able to elucidate the PD1 non responding a patients in a pretreatment biopsy.

NOTE Confidence: 0.876218438148499

00:17:36.890 --> 00:18:08.510 So now I'm going to transition into 2 pieces of unpublished work that I think this group might appreciate really kind of digging into the immune the Immunobiology of some of the deleterious populations that are present in the tumor. So what's so bad about hypoxia. We talk about it a lot and in fact, if you've been in the news. the Nobel Prize. Of course was awarded for hypoxia this week, but oxygen tension is this very interesting relationship with the immune system and that's if you take T cells and you just grow them.

NOTE Confidence: 0.86077207326889

00:18:08.510 --> 00:18:37.900 Up in the presence of hypoxia, they actually become great T cells. They're very good T cells. They kill targets very well. But it's all we know that hype. Oxy has been shown to do also have inhibitory pathways. So I actually put this up here hypoxia into both inhibit stimulate and have no effect on T cell function. If you look at the right if you look at the right papers, so sometimes it's with genetic systems, knocking out different

8

Alpha and not going out. VHL sometimes it's culturing cells under low oxygen tension or no oxygen tension, but it's a little bit unclear.

NOTE Confidence: 0.911130011081696

00:18:39.300 --> 00:19:10.710 But the important thing is that hypoxia in the tumor microenvironment is a little bit different. It's not just the Los of oxygen, but then the presence of deleterious byproducts right so as oxygen tension drops. You don't just lose oxygen you gain something bad, which is the production of reactive oxygen species. That's the first thing to talk about and as the cell becomes hypoxic. It also becomes hungry for glucose and as you breakdown in Ferment glucose. Yeah, you lose some glucose and that's important, but we also build up lactic acid.

NOTE Confidence: 0.857109010219574

00:19:10.710 --> 00:19:14.800 So those 2 things are the next thing to talk about hypoxia and acidosis in the tumor.

NOTE Confidence: 0.871991515159607

00:19:15.760 --> 00:19:47.310 So we hypothesize hypothese itself may not be a direct signal, but it changes the way that signals are received so I'm going to go back to some original data understanding what happens to T cells when they enter the tumor and what we found is that into moral T cells. A few years ago have very, very striking metabolic defects. This isn't Melanoma. B16 Melanoma bearing mice and were able to profile using a fluorescent glucose tracer attended the one that I talked about before this one called 2 NBDG and mitochondrial Maps with this die called Micro Tracker. You could find that T cells that infiltrate tumors.

NOTE Confidence: 0.877124011516571

00:19:47.310 --> 00:20:17.960 Very very striking metabolic defects and inability to take up this tracer and a loss of functional mitochondrial mass this occured this occured in many, many solid tumor models. We also saw it in human patient samples, which you can see up here. This is a Melanoma patients. We observe the same thing and head and neck cancer. Ovarian and lung is that when you look at a patients PBL versus the tumor infiltrating T cells. We could see see this mitochondrial atrophy that T cells.

NOTE Confidence: 0.896905183792114

00:20:18.010 --> 00:20:24.150 Lose their functional mitochondrial mass and that is actually also predictive of response to immune based therapies.

NOTE Confidence: 0.862694084644318

00:20:24.670 --> 00:20:55.160 So we linked this Los of functional mitochondria, with the ability of these T cells to persist and produce cytokine and what we found just again. I'm making a very, very Long story short so we can focus on the cool new stuff is that as T cells terminally differentiate to exhaustion and it's

a hot button word going to keep using it. This occured due to down regulation of this molecule called PGC when Alpha is the P part Gameco Activator 1A if you want to look it up.

NOTE Confidence: 0.903074562549591

00:20:55.160 --> 00:21:17.240 It is a transcriptional coactivator that program is a process called mitochondrial Biogenesis and as T cells differentiates from being PD1 low intermediate high and then becoming terminally differentiated they repress the expression of this molecule and that that was the smoking gun for why these mitochondria failed and that T cells lost some functional mitochondrial mass.

NOTE Confidence: 0.776232182979584

00:21:17.770 --> 00:21:18.920 And so.

NOTE Confidence: 0.896779119968414

00:21:19.500 --> 00:21:51.690 This has been very vexing to us and in my graduate student what she's now and she just got her. pH D but she wouldn't let it go. She couldn't. She didn't know whether or not T cells became exhausted and that made them metabolically insufficient or was metabolic insufficiency, causing the exhausted phenotype. She was obsessed with it. And here we are. So does encounter with metabolic stress contribute to the terminally differentiated States and So what happens when T cells differentiate.

NOTE Confidence: 0.843292415142059

00:21:51.690 --> 00:21:55.320 While there might, a conjurer being inactivated so to speak by hypoxia.

NOTE Confidence: 0.865370810031891

00:21:55.850 --> 00:22:25.960 So so here's what happens if you take if you take B16 Melanoma and you put it into mice and then you infuse them with him and it is all and again. Here's that same plots if you look at the degree of hypoxia in these cells using this. This PM and it is all antibody, which is showing us that they're not very good. I want to make sure that we're very clear the exhaustion field is a little hot button right. But the key point here with it is that exhausted T cells still do something they still make damn interferon their highly lytic.

NOTE Confidence: 0.878293335437775

00:22:25.960 --> 00:22:38.430 Are they are proliferating they just don't accumulate but what they do? Loses Poly functionality that ability to self? Renew and the ability to make many sided coin. So we're just doing this year with file to production, but here's the key points.

NOTE Confidence: 0.856705009937286

00:22:38.930 --> 00:23:08.970 Is that when you look at their hypoxia staining the PD one positive the Piedmont high temp 3 positive cells are uniformly

experiencing hypoxia. All of them are severely hypoxic and that's not the case with the other markers and you can also just staying for his 1A. It's not as dynamic and PM and it is all antibody, but it's showing us. The same thing. So exposure to hypoxia. We kind of reasoned, maybe part of the way that these cells differentiate 'cause is just so.

NOTE Confidence: 0.89691436290741

00:23:08.970 --> 00:23:39.370 It was a path these cells were on so we went and broke it down. I'm going to leave out some of the intermediate parts. But he wanted to understand what was that what was the experience of hypoxia in vitro doing to cells in isolation and so we identified ruled out a couple things we knew there was a rich literature about culturing cells in hypoxia, but that didn't take into consideration a main driver of the terminally differentiated phenotype, which is the continuous stimulation that occurs as T cells enter the tumor. So we asked a very simple question.

NOTE Confidence: 0.873033940792084

00:23:39.770 --> 00:24:10.480 If you have T cells experiencing hypoxia, but they're also getting other strong signals. What happens and that's that's what we did. We generated a model that was based originally in a coculture system with tumor cells. But, for the interest of time. I'm going to talk to you about this model where we activated T cells with off the shelf stimula. Tori beads overnights and then we either. Remove the beads magnetically or we kept that eat the beads in just kept them in Coculture with these anti CD 3IN CD 28 coded beads.

NOTE Confidence: 0.881460726261139

00:24:11.300 --> 00:24:42.030 But then we did that either at ambient normoxia 20%. Oh, two or in a hypoxia chamber. That's sent to 1.5%. Oh, two and we let that go and then we remove the beads and we expand the cells in aisle 2, Normoxia 'cause. We don't want to look at acute problems. We want to look at what happens after even after we remove that stimulus. This a model system. It's a model. It's not perfect, but one of the things that we that we found was that if the T cells are experiencing continuous stimulation or if they're experiencing hypoxia.

NOTE Confidence: 0.876638472080231

00:24:42.030 --> 00:25:12.260 You get these intermediate phenotypes, but but this is just PD one until 3 expression. But the experience of continuous activation in the presence of hypoxemia promoted a persistent upregulation of PD one and Tim 3, so most of the cells had this kind of terminally differentiated phenotype and I know most of you are thinking well. That's just kontora molecules. It doesn't mean anything so if you read nature recently. Everybody is excited about this molecule talks. This is a transcription factor that's associated with terminal exhaustion.

NOTE Confidence: 0.867158353328705

00:25:12.260 --> 00:25:19.090 And we can find indeed that if cells are experiencing continuous activation under hypoxia. They also up regulate this transcription factor talks.

NOTE Confidence: 0.876708745956421

00:25:19.680 --> 00:25:49.970 But but but the proof is really in the in the cytokine pudding and that is if you re stimulate those cells what we find is this just kind of beautiful. I love that kind of dichotomy of these data continuous activation. T cells are totally fine very good. T cells if T cells are culture in hypoxia. Previous consistent with previous literature. They get their fantastic T cells. They do very, very well. But the experience of both simultaneously promoted a Los of Poly functionality and a terminally differentiated phenotype.

NOTE Confidence: 0.884432256221771

00:25:49.970 --> 00:26:20.570 From a functional perspective, so that was really exciting so we have this idea that it was it wasn't just hypoxemia. It was the experience of lot of signaling in the presence of hypoxia, So what does that mean? What was actually happening and so we wanted to check our metabolic boxes off and so we can see lots of functional mitochondrial mass here with our might attract or Die. We can see repression of the PGC when Alpha molecule only when these cells are experiencing both a little bit when the T cells are continuously activated.

NOTE Confidence: 0.889786005020142

00:26:21.250 --> 00:26:38.270 And if we look in the seahorse instruments. We can see the red line is the continuous plus a hypothesis is that these cells have more of a flat line and no respiratory capacity so by all measurements. These cells looked exhausted like we're not going to call them exhausted, but they looked terminally differentiated.

NOTE Confidence: 0.836444318294525

00:26:38.870 --> 00:26:44.520 So exposure to hypoxia under conditions of continuous activation induced this persistent dysfunction.

NOTE Confidence: 0.874199867248535

00:26:45.140 --> 00:27:16.550 So what was actually happening. Now we had a system where we could decode the mechanism behind it, because it wasn't. The Dirty Environment of a tumor, but rather we had these discrete inputs and so one of the things that continuous activation upregulates. We know this from the literature is a pattern of transcriptional repression. That's driven at least in part by blink 1 very well known transcriptional repressor and you can see terminally differentiated T cells. But even the PD one intermediate's up regulates the expression of blimp one what we found is our in vitro system really drove.

NOTE Confidence: 0.845693171024323

00:27:16.550 --> 00:27:48.000 The expression of blimp on this, this transcriptional repressor such that the combination really had been highest expression of this and so we reason that maybe blimp might repress some of our molecules that we like and indeed that's what we found is that if you if you just take 293 T cells. For instance, and you overexpress blimp. You lose the protein of PGC when Alpha if you think this is a bad western block your right and but we did that we did. The real experiment, which is we used a PVC when Alpha promoter driving luciferase.

NOTE Confidence: 0.832571983337402

00:27:48.000 --> 00:27:52.730 And show that as you titrate in blimp you lose the activity of the PC when Alpha promoter.

NOTE Confidence: 0.804633975028992

00:27:53.230 --> 00:27:57.350 So we think that blimp is being turned on in that represses PGC went off.

NOTE Confidence: 0.858824610710144

00:27:58.530 --> 00:28:26.060 Now, if you have blimp deficient T cells. Those T cells are unable to be rendered dysfunctional in the continuous activation under hypoxia system. You can see this is double producers in awhile cipher block knockouts. But more importantly, blimp knockout. T cells in vivo don't have this Los of functional mitochondrial mass which is pretty exciting and they don't repress the expression of PGC when Alpha so we think that is a big driver of this process.

NOTE Confidence: 0.90407121181488

00:28:26.580 --> 00:28:57.370 So that now let us to believe? What does PGC when Alpha do? What does it actually doing that changes how we respond to hypoxia and so PDC when Alpha does a lot of things we like it in the lab because it does a lot of things it's not a master regulator. It's not like talks or Fox be 3 or something like that. It doesn't have any real effect on its own. But it collaborates with several different other important signaling pathways to orchestrates a whole degree of metabolic reprogramming and in the lab. We've looked at mitochondrial Biogenesis. We've looked at mitochondrial Fusion.

NOTE Confidence: 0.87879341840744

00:28:57.370 --> 00:29:13.000 But the one I'm going to talk about today is antioxidant activity. The fact that when you make you mitochondria. You have to load him up with things that are going to that are going to mitigate all of the reactive oxygen species that are going to happen that are going to be generated when the cell start doing oxphos.

NOTE Confidence: 0.889334559440613

00:29:13.620 --> 00:29:30.420 What we found is that we could stay in for mitochondrial reactive oxygen species using this die called my toe socks and you

could find as T cells terminally differentiate in Vivo. The upregulates they accumulate a lot of mitochondrial reactive oxygen species which you can see tabulated here.

NOTE Confidence: 0.891347229480743

00:29:31.020 --> 00:30:02.630 So that was that was an important thing to find so the key question is was that also happening in in our in vitro system and it absolutely was when cells were experiencing continuous activation under hypoxia. They started to accumulate mitochondrial reactive oxygen species. So this was this was important we wanted to understand a little bit more about what that reactive oxygen species was doing so. How can we do that under all of the things that we know hypoxia does so this is seemingly counterintuitive that hypoxia would induce reactive oxygen species because don't you need oxygen.

NOTE Confidence: 0.870589137077332

00:30:02.630 --> 00:30:32.750 But the fact is hypoxia is not an oxy Oh there is still oxygen present, and as oxygen levels. Drop you start to generate mitochondrial. Ross at complex. One this first step in the electron transport chain so how could we assess that in isolation is that delineating here is that we can actually use an old drug called Antimycin, A, which inhibits complex 3 at this place and when you do that. You mimic what hypoxia looks like without being hypoxic and that was able to generate Ross.

NOTE Confidence: 0.881278693675995

00:30:32.770 --> 00:31:03.440 It was really cool and I love these kinds of experiments is that antimycin a alone was able to generate mitochondrial. Ross and this has been known before you can use this as a Ross generator, but was really cool. If you could add another inhibitor wrote known and bring it back down to baseline so it rather than just simply neutralizing the raw so you can actually add a second inhibitor on Top of the first inhibitor and go back and rescue the phenotype so if you generate a lot of mitochondrial. Ross you generate exhausted T cells their terminally differentiated PD one Heights him 3 positive.

NOTE Confidence: 0.841810405254364

00:31:03.440 --> 00:31:05.170 And they don't make cytokines very well.

NOTE Confidence: 0.857069909572601

00:31:05.730 --> 00:31:16.710 This is an overnight activation with these molecules in case you guys don't believe me that were just poisoning the cells. They don't. They don't. They are acutely acutely toxic to the T cells, but rather changes the way they differentiate overtime.

NOTE Confidence: 0.900072395801544

00:31:17.840 --> 00:31:48.750 So it's not the Los This Is This Is This is a complete crazy set of experiments and it was years trying to get this stuff together is that it's not the Los of mitochondria. That's the problem because when you

inhibit with wrote Lonesome. I say you shut down the whole thing, but it's actually the presence of Ross that induces the dysfunctional states is not the loss of functional mitochondria is the accumulation of dysfunctional mitochondria that really drives. This process, So what was Ross actually doing. I know we're getting really deep into the rabbit hole here, but it's kind of needs one of the things that Ross does a lot of things.

NOTE Confidence: 0.859471499919891

00:31:48.750 --> 00:32:21.100 So we wanted to we wanted to drill down to what it might actually do and so one of the things that have been reported and we all know this 'cause you can add peroxide yourselves to to look at hyper phosphorylation and that's exactly one of the things that Ross does it actually inhibits phosphatases and so we thought that maybe intolerable levels of Ross might drive a kind of persistent phenotype a persistent tyrosine phosphorylation. So we just took the old 4G10 molecule that fossil tyrosine antibody that we used to use and we just stained T cells within kind of unsurprisingly T cells that are terminally.

NOTE Confidence: 0.862778604030609

00:32:21.100 --> 00:32:51.330 Terminally differentiated have high levels of tyrosine phosphorylation are being chronically activated in vitro. That's probably in Vivo. That's probably what's happening and the same thing happens in our continuous activation plot was really neat is that if you just do the answer lies in a thing just turn on Ross you get hyper phosphorylation of all the fossil tyrosines in the cell, which is pretty and so one of the one of the fossil tyrosines. Of course, there might be important. If you turn on a tyrosine phosphorylation. Cascade you may get and found in the nucleus, which we know of course.

NOTE Confidence: 0.860411167144775

00:32:51.330 --> 00:33:22.880 Is bad unless it is with other with a P1 for instance, you can drive exhaustion on its own and so kind of the idea behind all of this is that is that when you have this is intolerable levels of Ross. You're kind of keeping and found in the nucleus. All the time because you're hitting all your phosphatases and if you want to do a kind of a whacky experiment. You can actually culture cells in sodium North of antedate phosphatase inhibitor. It's not very good for cells. But the cells that does leave behind have a terminally differentiated phenotype.

NOTE Confidence: 0.759774148464203

00:33:22.880 --> 00:33:26.860 Of being PD 1 high temp 3 positive and turning on blimp in talks so.

NOTE Confidence: 0.878742516040802

00:33:27.460 --> 00:33:39.530 I know it's a lot to that's a lot to see but continuous stimulation represses the machinery needed to. To mitigate hypoxia induced

Ross' and that results in this kind of pathologically elevated phosphorylation Cascade.

NOTE Confidence: 0.891582071781158

00:33:40.040 --> 00:34:10.270 So does this matter, so remember that RNA interference. I told you before the we knock down the complex one. What was really cool is the reason why we used RNA interference is because you can RNA interference is reversible crisper. You can't come back from a deletion right so we were able to actually generate tumors that had a docs inducible RNA interference, so the tumors are totally wild type and then we can render them less oxidative in vitro and here and in vivo that 48 hours of going on doctor cycling.

NOTE Confidence: 0.854586541652679

00:34:10.270 --> 00:34:14.420 Turns on this RNA interference and it renders the tumors less hypoxic.

NOTE Confidence: 0.83592689037323

00:34:15.160 --> 00:34:36.860 And so here's the T cell hypoxia. If we stayed for payment is all by flow and so it was really neat is that if you have the mouse and you don't give it docs. It's metabolically wild type and PD. One doesn't work. But if you then add dockson give anti PD. One interrupt that hypoxia. You're then able to get a really striking synergy and most of them clear their tumors with anti PD one.

NOTE Confidence: 0.904901504516602

00:34:37.730 --> 00:35:08.550 So this, this model of kind of metabolic exhaustion is that the persistent stimulation drives a pattern of transcriptional repression that changes the way that you deal with hypoxia and so it's kind of a mix of two things is that persistent stimulation changes the way you deal with hypoxemia and hypoxia feeds forward and changes the way that you experience other signals and one of those mechanisms is by fossil taste. In addition, however. I'll tell you that there's a lot of other things that Ross does and we're excited about epigenetic remodeling.

NOTE Confidence: 0.810340642929077

00:35:08.550 --> 00:35:13.650 In lipid storage changes that happen in in response to Ross.

NOTE Confidence: 0.81522125005722

00:35:14.500 --> 00:35:16.020 OK, that's fine.

NOTE Confidence: 0.871005415916443

00:35:17.670 --> 00:35:49.800 So sorry just like the second time I've given that that that story, so our lab is really excited about metabolic changes of immunotherapy and I think the cool part about it is that Mataba Lism is so central to biology and it's such a central defect and anti tumor immunity that

there is a lot of axes that you can change, so one of the things we're interested in courses in adoptive cell therapy and how do you metabolically change adoptive cell therapies like put in PC in Alpha back into T cells like into car T cells for instance, which is something we're excited about how do we change immune excluded tumors?

NOTE Confidence: 0.834797978401184

00:35:49.800 --> 00:36:20.980 These cells that are there, but they're not in the tumor bed. We think hypoxia medication may be a great place to do this we have a clinical trial of using metformin, which is a complex one inhibitor. It's an inhibitor of that molecule that we knocked down in the in combination with anti PD one. We have a lot of excitement about using TFR stimulators like 4 would be an OX40 agonists. Those we have linked to mitochondrial reprogramming T cell and in cases of immune desert tumors. I don't think we're even out.

NOTE Confidence: 0.907021582126617

00:36:20.980 --> 00:36:32.980 Out of the Woods, yet because we recently reported that you can actually stimulate the immune system with an uncle. Dick virus and then encode metabolic reprogramming in the virus itself, which is brand new but all of these things.

NOTE Confidence: 0.824188768863678

00:36:33.530 --> 00:37:04.580 Are focused on the TSLCDAT cells? We've been excited about CDT sells for awhile there cool? But with all of the focus. The fact of the matter is the majority of T cells in the tumor are not CDC sells their regulatory T cells. There tall orogenic T cells. So this is where I did my postdoc studying regulatory T cells and this is how my lab got started but it took us a long time because Fox be 3 reporters takes forever to breed so T Rex cells are these highly overrepresented T cells in the tumor.

NOTE Confidence: 0.86143285036087

00:37:04.580 --> 00:37:35.750 You can see that this isn't a lymph node. There's very small proportion of T cells right. But in the tumor. The majority of T cells are regulatory but they're not just idly standing there right there actively proliferating right. Most of them are Ki 67, positive and a good proportion of the cases at 7 positive cells are in S phase so this environment that I told you is so bad so toxic see work cells are fine. There so why or T Rex. I'll still find there is something that I've really been struggling with since I started my lab, you can get rid of T Rex.

NOTE Confidence: 0.864000976085663

00:37:35.750 --> 00:38:06.510 But it's not good right T Rex else if you get rid of T. Rex else with with a genetic system. The tumors melt away but then the mouse dies from autoimmunity. So you can't just get rid of T. Rex have to change them and so we kind of hypothesize the T Rex else must have a metabolic profile. That's distinct that allows them to thrive in the tumor. And

if we could identify what that metabolic profile was we could target and then maybe only take care of T. Rex cells that are in the tumor, so how do we do this?

NOTE Confidence: 0.819204092025757

00:38:06.510 --> 00:38:13.740 We use the same glucose tracer that we had before called 2 NBDG you can infuse it into mice. But these mice are Fox P3 Reporter notes.

NOTE Confidence: 0.88683158159256

00:38:14.300 --> 00:38:44.550 And consistent with data that came from several groups tier egg cells. Unlike their conventional counterparts hate glucose they won't take up the tracer. Even if you bathe them in that race are they really do not like taking up glucose. There's a subset that do this small proportion. So we hypothesize. Maybe those are important. Now it was really cool is that this tracer is alive cell tracer and this is a Reporter. So we can actually sort out cells based on their metabolism. And then assess them functional and unlike CD 8 cells.

NOTE Confidence: 0.838037610054016

00:38:44.550 --> 00:39:13.910 But you can just look for cytokines. T Rex else there. Um suppressive activity. You have to measure indirectly So what we do is you could sort out the T red cells based on their metabolism. And then just coculture that's called a suppression asset. You coculture them with proliferation die labeled conventional T cells. So if you don't have any T Rex. This is you can see the dye dilutes. It was really exciting was the 20 red cells did take up glucose that small proportion of them that did take up glucose they were actually not very good T Rex.

NOTE Confidence: 0.830887258052826

00:39:14.410 --> 00:39:45.390 And it was actually these metabolically inactive these less glucose avid T regs that were fantastic. T Rex else. So what does that mean so we took out those cells and because then they were alive, we could sort them out based on the mataba lism weeded RNA sequencing on these samples and it was exciting about this is that NBG low T Rex. So the less glucose. Abate ranks, they were fantastic T regs and at the high T Rex, the ones that took up this tracer. They were they weren't unstable they didn't stop becoming T Rex.

NOTE Confidence: 0.849887132644653

00:39:45.390 --> 00:40:16.430 They were just a little bit less like T Rex so all of these T Rex Signature Jeans were lower in glucose avid T. Reg cells with the exception of a few with notable exceptions, so we then went to the RNA sequencing and said, so they look a little bit less like T regs, but can we get a sense about what kind of metabolism. They were doing so, we went to look at

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a bunch of metabolic jeans and because I'm running out of time and you guys wanna hear me talk forever going to focus on these 2 jeans.

NOTE Confidence: 0.87232893705368

00:40:16.430 --> 00:40:48.260 Glycolysis was interesting Lee all over the map even though those cells didn't take up glucose so all these jeans. These are all the jeans involved in glycolysis, but the NBG low tier egg cells upregulated 2 jeans very significantly. LDH and this molecule S LC1681, all the SLC jeans usually people throw away but they're actually very important what we found is that if you take to your egg cells and you just culture them in low glucose conditions. They up regulate these things this molecule S LC1681, and LDH the terminal steps of glycolysis.

NOTE Confidence: 0.890848517417908

00:40:48.280 --> 00:41:19.150 And if you remember from those undergraduate biochemistry lectures that you probably forgot about glycolysis is reversible right that like house is not a one way streets. All of those steps are reversible with with a few notable exceptions, so in fact, this molecule, which is called MCT one or mono carboxyl ate transporter one transports. Mono carboxylates and one of the most highly prevalent. Mono carboxylates in the tumor is lactic acid. The byproduct of all that Furman Tate if.

NOTE Confidence: 0.833202421665192

00:41:19.150 --> 00:41:29.440 Glycolysis that we were just that I was alluding to in fact, in areas of the tumor. The lactate concentration get as high as 50 millimolar. But if you just squeeze a tumor and measure the average lactaid about 10.

NOTE Confidence: 0.884318947792053

00:41:30.210 --> 00:42:01.360 So this is a really important we think that causes a one way streets. But in fact, if you may remember things like the Cori cycle or the fact that you're nervous system runs on Lactates is that most of the enzymatic reactions are reversible and they can interconvert and so we hypothesize that lactic acid may be utilized to fuel by T. Rex cells and it occurs to this molecule. MCT one that forms a hetero dimer with CD 147, the only reason I bring it up is it before there were CD25C. One 47 was thought to be the marker for T Rex cells in humans.

NOTE Confidence: 0.856790065765381

00:42:01.430 --> 00:42:04.880 So we hope so. We were pretty excited about what this might mean.

NOTE Confidence: 0.851374566555023

00:42:05.410 --> 00:42:06.810 And then it can be converted in used.

NOTE Confidence: 0.902788519859314

00:42:07.410 --> 00:42:17.950 And so it was really exciting. We know lactic acid is an immunosuppressive molecule if you take conventional T cells and you put them in high concentrations of lactic acid. They slow down. We've done that for a long time.

NOTE Confidence: 0.873978853225708

00:42:18.470 --> 00:42:49.680 But if you take tier egg cells and put them in high concentrations of lactic acid. They tolerate it. In fact, they might even like it and they they are able to hang on to these various set conditions and they don't lose their function by being by being in the presence of lactic acid, so being around lactic acid was important, but where they actually utilizing it and we utilized in a old school assay were able to measure the pH changes intracellularly that are induced by lactic acid and we could find the T Rex else uniquely able to be acidified.

NOTE Confidence: 0.853965222835541

00:42:49.680 --> 00:42:58.400 When you treat them with lactic acid, so they're able to take it up. And if you look at the cells that take up lactic acid. They look like great T Rex, the express CD 44 and new repellent.

NOTE Confidence: 0.897813081741333

00:42:59.350 --> 00:43:20.780 So we then had to do real metabolism, which was trace lactic acid and to make a very Long story short what I will. I will tell you is that we are able to show that lactic acid? Does get efficiently taken up by regulatory T cells and interconverted into TCA cycle intermediates, but it also is utilized in higher higher order intermediates In other words.

NOTE Confidence: 0.862080931663513

00:43:21.530 --> 00:43:29.410 They are utilizing lactate in their utilizing the carbon. They're not just burning them. They're utilizing the carbons to support there to support their biology.

NOTE Confidence: 0.86368989944458

00:43:31.580 --> 00:44:03.470 So what are the requirements? What is the requirement for lactic uptake in vivo So what we did is we obtained in collaboration with Jeff Rothstein at Hopkins report sorry a flock still legal for that. MCT one molecule that molecule that we found was upregulated in T. Rex size if you delete that transporter the cells can't take a black dates, but in the periphery in the lymph nodes. Everything is fine. the T Rex sells the mice don't get sick and the T red cells are able to suppress if we put them into its suppression essay.

NOTE Confidence: 0.881200730800629

00:44:03.470 --> 00:44:33.790 But if we then challenge the mice with a tumor. We have this very slow indolent tumor growth so in that lactate rich environment. the T Rex else get in and they stopped working the CDA T cells in the tumor. Microenvironment proliferate more. The conventional cells do and they're very,

very good cited kind producers. They're double producers of CNF and amateur fear on, but the T. Rex cells themselves, they start destabilizing they're unable to support the rapid proliferation. We were talking about and more importantly, when we put them into a suppression assay they lose the ability to suppress.

NOTE Confidence: 0.877843201160431

00:44:33.790 --> 00:44:44.340 Conventional T cells and they even start making Gamma Interferon, so this part. This lactic acid is not just a fuel source. But it's helping support that the biology of T Rex Cells.

NOTE Confidence: 0.873840570449829

00:44:45.520 --> 00:45:15.680 All right there's a lot of information, but I want you guys to be excited metabolism. So the lab is really excited about about lowering metabolic barriers to anti tumor immunity that could be bolstering the T cell providing metabolic supports totuma reactive T cells that could be reprogramming the tumor. Changing the way that it does metabolism to re to re balance the environments. But we can't forget about these guys that there are immunosuppressive relationships that are formed that utilise une appreciated metabolic pathways.

NOTE Confidence: 0.845805704593658

00:45:16.270 --> 00:45:38.910 With that I'd like to thank the people in the lab that did the work Plaid is our uniform in the lab Nicole drove the hypoxia stuff. Mac is driving the acidosis stuff and Ashley did the tumor cell metabolism stuff we couldn't do this without fantastic collaborations with Amanda Pollak, who's a blimp biologist working pits and Jeff frosting for the animals happy to take any questions and sorry if I went over.

NOTE Confidence: 0.883712410926819

00:45:51.330 --> 00:46:21.540 I knew I was going to regret not paying attention to my metabolism course in medical school I just notice 2 things so one was in the anthem meissen treated cells. You had a picture where actin seem to be change its distribution. But you didn't mention anything about why you show that in the 2nd question is I also.

NOTE Confidence: 0.879338145256042

00:46:21.700 --> 00:46:53.280 Noted that EB I3 was very particularly high in the T Rex that weren't uptaking glucose and the question. There was Do you CP 35 as well? Yeah. Great question so the first thing is the act in in that experiment. I think was just to show you what the cytoplasm look like versus the nucleus is kind of showing the localization. However, we do know that in all honesty cytoskeletal rearrangement is extraordinarily metabolically demanding.

NOTE Confidence: 0.8315549492836

00:46:53.280 --> 00:47:23.460 And so one of the things we're studying the lab is are the metabolic pathways that lead to good motility, but still in its infancy

in the 2nd that your 2nd question is, is I think right dead on so P-35 is difficult to detect we've Darius talked about this quite a bit we did. We do see slight signature for P-35 in actually in the highly glycolytic T. Rex else so that's an interesting thing actually is that the glucose avid T Rex else aren't.

NOTE Confidence: 0.804445564746857

00:47:23.460 --> 00:47:45.170 Are still T Rex? But we think that they suppress by different mechanisms than uh than the non glucose Alpha T Rex. But we're still measuring. I'll 35 coming out at T Rex is still tough to to do. But we do have a by 3 reporters as well as P-35 reporters now in our lab, so we can do something we can explore.

NOTE Confidence: 0.853595912456512

00:47:46.900 --> 00:48:18.800 Yeah, lactate in the tumor microenvironment is can affect a couple different cell types and uh so Oscar Kaleo and Russell net stops lab a couple years. Back had shown that tumor associated myeloid cells or macrophages utilized lactate as well. An uptake that so any thoughts about that contribution potentially in the tournament. This is I really want to talk about lactate here for that reason because I think was very. I think what we're finding is that many immunosuppressive cell types utilized these alternative sources of fuel.

NOTE Confidence: 0.896434783935547

00:48:18.800 --> 00:48:48.500 And lactate is just one of the things actually that the that MCT one. It's a very promiscuous. Transporter so transports all kinds of things, including short chain fats like Butyrates and ketone bodies as well. So all of these but these are all fuel sources that are very prevalent in the tissues. And so we think that this is a phenotype of being able to survive and suppressing the tissues and I think that the story and tumor associated macrophages. I think is part and parcel of that.

NOTE Confidence: 0.87134450674057

00:48:49.010 --> 00:49:21.000 Would MCT 1 as a potential pharma pharmaceutical target be too likely to have toxicity elsewhere? Do you think or are you thinking about that at all actually so there's several so they were empty one inhibitors an antibodies have both been tried in the clinic in the days of just trying to starve tumor cells to inhibit glycolytic tumor cells. So many of these agents are now being repurposed in combination with immune based therapies. We have utilized both AM CT12 selective inhibitor.

NOTE Confidence: 0.851824522018433

00:49:21.000 --> 00:49:32.000 In combination with adoptive cell therapy and anti. PD one in mouse models and we see a very nice synergy. But it's tough to drill down on what is actually a T Rex phenotype versus changing the micro environments hole?

NOTE Confidence: 0.890687763690948

00:49:33.770 --> 00:50:06.300 Those fantastic took what I'm wondering about is how much of this specially this switch to this exhaustion phenotype in the mailbox, which how much of that do you think is due to need to survive and you're taking away certain things away from those cells versus versus actually like programming driving the process. Yeah, I think I think it's a fantastic question. But I think it's driven in part by kind of trying to reform how we think about the terminal exhaustion.

NOTE Confidence: 0.89271491765976

00:50:06.300 --> 00:50:29.880 Phenotype writes that terminally differentiated phenotype is inherently a stressful pathological response and so I think many of these things are driven by the fact you suppress PC when Alpha because you need to do something right and even though it's pathologically amplified. The fact is we know that this continuum exists for a reason right that you drive to terminal differentiation for a reason. And So what we're trying to explore now is what are the?

NOTE Confidence: 0.884136259555817

00:50:30.390 --> 00:50:57.760 You know what are the survival type phenotypes because metabolic reprogramming is of course important for all cells not just T cells but then being able to assess? What are the individual pathways that contribute to that that self sufficiency or lack of self sufficiency and is that just what happens is you differential use. It become more depending on your environment. So I think it's a multifactorial question, we're very excited about what those things might mean but don't all the answers yet.

NOTE Confidence: 0.821939468383789

00:50:59.940 --> 00:51:02.520 Great, thanks very much.